Trichomonas vaginalis origins, molecular pathobiology and clinical considerations

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Abstract:

Purpose of review: We aim to integrate a selection of the most recent data on *Trichomonas vaginalis* (TV) origins, molecular cell biology and TV interactions with the urogenital tract microbiota with trichomoniasis symptoms and clinical management.

Recent findings: Transcriptomics and proteomics datasets are accumulating facilitating the identification and prioritisation of key target genes to study TV pathobiology. Proteins involved in host sensing and cytoskeletal plasticity during TV amoeboid transformation were identified. TV was shown to secrete exosomes and a macrophage migration inhibitory factor-like protein that both influence host-parasite interactions. TV co-infections with *Mycoplasma* species and viruses were shown to modulate the inflammatory responses while TV interactions with various *Lactobacillus* species inhibit parasite interactions with human cells. TV infections were also shown to be associated with bacterial vaginosis (BV). A broader range of health sequelae is also becoming apparent. Diagnostics for both women and men based on molecular approaches are being refined, in particular for men.

Summary: New developments in the molecular and cellular basis of TV pathobiology combined with data on the urogenital tract microbiota and immunology have enriched our knowledge in human-microbe interactions that will contribute to increasing our capacity to prevent and treat TV and other STIs.

Keywords:

Omics data, *Mycoplasma*, *Lactobacillus*, *microbiota*, Trichomonasvirus.

Abbreviations:

Extracellular matrix (ECM); lipophosphoglycan (LPG); MIF macrophage migration inhibitory factor; nucleic acid amplification tests (NAATs); sexually transmitted infections (STI); tetraspanin (TSP); *Trichomonas vaginalis* (TV); *Trichomonas tenax* (TT); *Trichomonas vaginalis* virus (TVV); vaginal epithelial cell (VEC).

Introduction

Trichomonas vaginalis (TV) is the most common non-viral sexually transmitted infection (STI) worldwide. The World Health Organisation estimated that in 2005 there were 248 million cases of TV and this had increased by 11.2% to 276.4 million cases in 2008 [1]. There is significant regional variation with the highest rates in the Americas with an incidence of 177.7 per 1000 females and 180.6 per 1000 males aged 15 to 44 and a prevalence of 22% in females and 2.2% in males. This contrasts with South East Asia where the incidence is estimated at 40.3 per 1000 females and 50.1 per 1000 males with a prevalence of 5.6% and 0.6% respectively. Notably TV is affecting primarily woman of disadvantaged populations in both affluent and resource limited countries and is still very much a neglected disease despite increasing awareness of its important health sequelae.

The availability, for one TV strain, of a draft genome sequence and annotation [2] have stimulated numerous studies on the parasite population genetics and molecular basis of it pathobiology [3, 4]. These developments paralleled with studies on the urogenital microbiota and immunology will eventually converge to improve our knowledge of TV pathobiology and assist with prevention and treatment protocols. Here we present an initial integration of a selection of the most recent developments in basic research on the parasite molecular cell biology with its clinical symptoms and management.

TV as a bird derived zoonosis?

A number of different molecular phylogenies have surprisingly supported a close relationship between TV and parasites isolated from birds [5]. The two human parasites TV and *Trichomonas tenax* (TT, from the oral cavity) were thought for some time to be closely related to, and possibly derived from, each other [2, 5]. Molecular phylogenies established that TV and TT are more closely related to distinct species of *Trichomonas* isolated from birds then they are to each other (Figure 1). This suggests two independent zoonotic origins from birds for these two human parasites. Hence it will be of great interest to perform comparative studies of the genomes and host-parasite interactions between human and bird *Trichomonas* species to identify the molecular basis

of their respective pathobiologies. Thus TV is likely join the long list of human pathogens derived from birds [6]. These considerations further illustrate the importance of studying pathogens in an integrated way across the fields of medical and veterinary research [6].

Genomics and allied omics

The draft genome sequence data and annotation for one clinical TV strain uncovered a surprisingly large and highly repetitive genome for a microbial eukaryote (Table 1) [2, 4]. These data represent an invaluable source of information to study the epidemiology and molecular cell biology of host-TV interactions [3]. Only a selection of recent developments is discussed here and complementary reviews cover a broader range of issues [3, 4, 7-9]. Due to TV vast potential protein coding capacity (Table 1), transcriptomics and proteomics investigations are particularly pertinent to identify and prioritise the functional characterisation of key TV proteins enabling infections [3, 4].

Two recent studies have initiated the global profiling of TV gene transcription in various *in vitro* growth conditions for two distinct TV strains using RNA-seq methodologies [10, 11]. Circa 30,000 genes were transcribed in the various tested conditions in each strain [10, 11] and a core set of about 20,000 genes was identified across the 11 conditions considered in one study [10].

Modulation of mRNA levels upon exposure to oxidative stress, binding to vaginal epithelial cells (VEC) or glucose restriction have identified hundreds of genes involved in responses to these environmental cues. These included genes mediating oxidant scavenging and cytoskeletal re-arrangements upon exposure to oxygen, binding to VEC or a combination of both [10], or amino acid metabolism upon glucose restriction [11]. Notably the combined exposure of TV to oxygen and VEC stimulated the broadest set of TvBspA genes up-regulation [10], consistent with the hypothesis that the largest gene family (911 genes) encoding candidate surface proteins are important in host-TV interactions [12]. A selection of TvBspA genes are listed in Supplementary digital content 1 (SDC1), illustrating the striking variation of transcription for some genes between the two studied strains, possibly underlying antigenic variation [7, 12]. The modulation of the actin-based cytoskeleton upon contact to VEC at the transcription level was also highlighted [10]. TV trophozoites

binding to VEC rapidly differentiates into an amoeboid form maximising contact with host cells, a key step in the infection process (Figure 2). The fimbrin protein (an actin bundling protein) was recently shown to dramatically change its cellular distribution from the cell periphery in trophozoites to focal points co-localising with actin in the amoeboid form [13].

With a highly repetitive genome possessing numerous transposable elements (TE), it is important to highlight here the recent analyses of one family of TE (class II Tc1/mariner) and their impact on gene transcription across 94 TV isolates [14]. Several insertions of TE were demonstrated to reduce or abolish the transcription of neighbouring genes, suggesting that such TE driven mutations could have functional implications and contribute to adaptive evolution of the parasite [14]. Combining comparative genomics and transcriptomics across a number of strains will be required to assess the role of TE in mediating TV genetic and phenotypic diversity.

An annotated genome also facilitates proteomics investigations and a number of such datasets have been published [3, 7, 9]. Here we consider the most recent data on surface proteins and cargo proteins of the secreted exosomes.

Surface and secreted proteins and exosomes

Surface and secreted proteins from the parasite are at the forefront of host-parasite interactions; hence these proteins have attracted great interests [7-9]. Two recent insights into the molecular basis of host-parasite interactions are considered here.

Gene annotations identified a total of 11 TV tetraspanin (TSP) proteins possessing the characteristic four transmembrane domain of TSPs and two strains transcribe slightly different set of these genes (SDC1). Based on the numerous role of TSP proteins in human and model systems in orchestrating cell adhesion, fusion, signalling, proliferation and migration and their status as markers of exosomes, identified TvTSP as important target proteins to study host-TV interactions [15, 16]. Cell surface proteomics confirmed three TvTSP proteins [15] and one TvTSP protein, TvTSP6, was functional investigated [16]. The TvTSP6 cellular re-distribution (from flagella to the main cellular body and large intracellular vesicles) upon TV binding to VEC and its role in regulating TV migration, makes it a prime target to further investigate how TV

sense and orchestrate its binding to, and migration through, human tissues during infection [16]. The TvTSP6 and the fimbrin re-localisation data [13] illustrate the dramatic cellular changes taking place in TV upon binding to host cells.

The secretion of exosomes by TV was investigated through a combination of cellular and proteomics approaches and identified exosomes with cargo RNA and a total of 215 cargo proteins including TvTSP1 and TvBspA_TVAG_216280 [17], the most and second most highly transcribed genes of these two gene families, respectively, in the recent RNA-Seq data (SDC1). Remarkably, TV exosomes were shown to bind to host cells, to modulate the TV-TV binding and TV-VEC and TV-prostate cells binding properties and to have immunomodulatory properties making these microvesicles and their cargo proteins attractive targets to gain new insights into TV pathobiology [17]. These various data identify the two exosomal membrane proteins TvTSP1 and TvBspA_TVAG_216280 as potential antigens for vaccine developments [18].

Another secreted factor likely to be important in modulating host-TV interactions is the TV macrophage migration inhibitory factor (TvMIF_ TVAG_219770) that was recently shown to be similar to human MIF and functionally investigated [19]. Notably the TvMIF gene is highly transcribed whereas a related pseudogene is not (SDC1). Functional characterisation of the TvMIF protein demonstrated that it was active in inhibiting macrophage migration, stimulating host cell signalling, was interacting with the human CD47 MIF receptor and increased the growth and migration through matrigel of both benign and prostate cancer cells [19]. The data also demonstrated an adaptive immune response to the TvMIF in TV positive patients, consistent with the protein being secreted during infections. Could different TV strains express variant TvMIF genes in response to this host response? If so this might rationalize the TvMIF pseudogene (SDC1). Taken together these data are consistent with TvMIF stimulating inflammation and host cell proliferation and could be responsible for the underlying TV association with aggressive prostate cancers [19].

TV-microbes interactions and their impact on host-TV relations

It is increasingly recognised that studying host-parasite-bacteria-virus complex interactions at mucosal surfaces represent an important new paradigm to comprehend the impact, positive and negative, of these interactions on human health and disease conditions, and TV is no exception [20](Figure 2). Three recent developments are of interest in this context.
First TV interactions with *Mycoplasma hominis* have been shown *in vitro* to synergistically up-regulate macrophage pro-inflammatory responses [21]. The pro-inflammatory responses to the TV-*Mycoplasma* consortium on immune, and possibly epithelial, cells might be clinically relevant by contributing to various health sequelae associated with TV infections (see later sections).
Notably a recent metagenomics investigations of the vaginal microbota led to the characterisation of the genome sequence of a new species of *Mycoplasma* that is strongly associated with TV infections [22]. This suggests that different TV-*Mycoplasma* consortia might exist. Could these lead to variations in inflammatory responses [21] and variations in TV gene transcription and protein synthesis profiles?
Second, endogenous TV viruses (TVV) isolated as TV-TVV consortium from symptomatic female patients were also shown *in vitro* to stimulate pro-inflammatory responses in human VEC [23]. Notably metronidazole treatments amplified the in-vitro pro-inflammatory responses in TVV positive parasites possibly due to the increase in TVV particles released from dying parasites. This phenomena could explain the negative impact of drug treatments observed during pregnancy [23](see later sections).
Finally, the effect of different Lactobacilli in *in vitro* TV-VEC interactions assay was investigated and demonstrated that the bacteria mostly associated with healthy vaginal microbiota inhibited these interactions, consistent with protective functions of Lactobacilli species, with the exception of *Lactobacillus pentosus*, which enhanced TV adhesion to VEC [24]. These observations were shown to be Lactobacilli dose dependent [24]. Consistent with these data TV infections were shown to be associated with bacterial vaginosis, a dysbiosis characterised by the loss of protective Lactobacilli [25, 26].
These different considerations suggest that diagnostics will need in the future to integrate TV, bacteria (mutualists and pathogens) and viruses (from both TV and humans) involved in the complex microbial ecology of the human

urogenital tracts, possibly by adapting metagenomics approaches currently exploited to characterise the complex host-microbiota-pathogens interactions e.g. [22]. So for example differentiating TV infections from TV-TVV, TV-Mycoplasma or TV-TVV-Mycoplasma co-infections could benefit patients by pointing to differential treatments minimising inflammatory sequelae in those different contexts [23].

Impact on human health

Previously regarded as a nuisance for women, it is now recognised that TV has a number of presentations in both genders, is a risk factor for HIV transmission and acquisition, as well as being associated with adverse pregnancy outcomes. TV infects the squamous epithelium of the genital tract, often including the female urethra. The usual symptoms of TV are vaginal discharge in women associated with vaginitis, cervicitis, and urethritis, although it is recognised that up to 1/3 women have no symptoms [27]. Recent studies have suggested an increased risk of acute endometritis in association with TV [28]. In men, TV is usually a transient infection and studies suggest up to 75% of men are asymptomatic but it may be associated with urethritis, rarely with balanitis [29] and possibly prostatitis [30]. A number of case reports suggest a widening of the pathologic spectrum of TV in humans including vulval ulceration [31], adult conjunctivitis [32] and pulmonary infections in association with HIV [33] and there is discussion around the possible contribution of TV to aggressive prostatic cancers [19, 34].

TV in pregnancy has been associated with low birth weight, premature rupture of membranes and preterm delivery [35, 36]. However some studies have failed to show that treatment of TV improves pregnancy outcome and some have indicated that treatment of TV infection in pregnancy may have a negative impact on the pregnancy [37, 38] - possibly explained by TVV inducing strong pro-inflammatory response upon drug treatment discussed earlier [23]. Neonatal infection, from vertical transmission with TV is rare. It usually causes a urogenital infection and there are case reports of vulvitis and

positive urine cultures in neonates. There are occasional reports of TV causing neonates respiratory tract infection [39].

Multiple reports support an epidemiological association between HIV and TV. There is growing evidence that TV infection may enhance HIV transmission and acquisition and there may be an increased risk of TV infection in those that are HIV infected [40-42]. In women with vaginal TV who are HIV positive there is some evidence that TV may be more difficult to treat. A randomized clinical trial found that a 2g single oral dose of metronidazole was not as effective as 500mg of metronidazole twice daily for 7 days for trichomoniasis among HIV-infected women [43].

Diagnostics

The choice of specimen, diagnostic test and testing strategy for detection of TV will depend on the patient population, clinical setting, and the available laboratory facilities. In settings where there is a high prevalence of STIs nucleic acid amplification tests (NAATs) are now the test of choice where resources allow and have become the 'gold standard' offering the highest sensitivity for the detection of TV [44-47]. In settings where molecular testing is not available the diagnosis of TV infection remains problematic because wet mount microscopy alone performs poorly for the diagnosis of trichomoniasis in women. Wet mount microscopy and current rapid antigen detection tests are not suitable for diagnosis of trichomoniasis in men.

A number of commercially available NAAT assays have sensitivities of 95–100% and the specificity is also 95–100% depending on the specimen and reference standard. The APTIMA TV Assay (Hologic Gen-Probe, San Diego, CA) was FDA-cleared in 2011 for use with urine, endocervical and vaginal swabs, and endocervical specimens collected in the Hologic PreserveCyt solution (ThinPrep) from females only. The BD ProbeTec Trichomonas Vaginalis Qx Amplified DNA Assay (Becton Dickinson, Franklin Lakes, NJ) launched in Europe (EU cleared) in 2012. The diagnosis of TV in men has been challenging given the low sensitivity of microscopy and lack of FDA clearance to date for any NAATs or point-of-care tests for use with male

specimens. Some laboratories have verified the performance characteristics of NAATs through a validation process for male urine specimens or penile-meatal swabs [45].

Culture, until recently, has been considered 'the gold standard,' with specificity approaching 100% but the sensitivity can be as low as 75% compared with molecular testing. Culture systems such as InPouch TV (BioMed Diagnostics, San Jose, CA) allow for direct inoculation, culture and microscopic examination. Such systems are useful when immediate transportation of specimens to the laboratory is not available. The specimen should be inoculated within an hour of collection to maintain viability of the parasite [46].

A number of point of care tests that detect TV antigens or nucleic acids have been recently developed that allow for extended time between specimen collection and testing and more flexible sample storage temperatures. Commercially available tests include the OSOM Trichomonas Rapid Test (Sekisui Diagnostics, California, USA) and the TV latex agglutination test (Kalon Biological, Surrey, UK) and the Affirm VPIII (Becton Dickinson, Maryland, USA). The sensitivities of these tests are similar to culture and consistently higher than wet mount microscopy. The specificities range from 92% to 100%. All three tests are intended for use with vaginal swabs from symptomatic women. However, none has been evaluated for screening asymptomatic women or for the diagnosis of TV in men.

Traditionally the diagnosis of TV has been made by the detection of the motile parasites on a wet mount preparation of vaginal or urethral secretions by light-field microscopy. Microscopy has the advantage that it is cheap, and can be performed near to the patient in a clinic setting. The specificity with trained personnel is high. However specimens should be examined within 10 minutes and the sensitivity is reported to be as low as 45-60% in women and even lower in men.

Treatment

Treatment for TV is with nitroimidazoles. As TV in women frequently infects the urethra and paraurethral glands and cure rates of around 50% with metronidazole intravaginal gel are reported, systemic treatment is required. A Cochrane review found a parasitological cure in >90% of cases of TV with almost any nitroimidazole drug given as a single oral dose or over a longer period [48]. Single dose treatments are associated with more frequent side effects than longer oral treatment. Standard treatments are a 5 to 7 day course of metronidazole 400 to 500 mg twice daily or 2g of metronidazole or tinidazole as single dose [49, 50].

Meta-analyses have concluded that there is no evidence of teratogenicity from the use of metronidazole in women during the first trimester of pregnancy [51]. Metronidazole can be used in all stage of pregnancy and during breastfeeding. Metronidazole enters breast milk and may affect its taste. The manufacturers recommend avoiding high doses if breastfeeding or, if using a single dose of metronidazole, breastfeeding should be discontinued for 12-24 hours to reduce infant exposure. Tinidazole is pregnancy category C (animal studies have demonstrated an adverse event) and its safety in pregnant women has not been well-evaluated.

Persistent or recurrent TV may be due to inadequate therapy, re-infection, or resistance [52]. Development of resistance against nitroimidazoles can be due to aerobic and anaerobic resistance. In the USA, it is estimated that 5% of clinical isolates of TV exhibit some degree of metronidazole resistance, predominantly low level [53]. In vitro resistance does not predict clinical response to treatment, as it may be relative rather than absolute, and may be overcome by high dose metronidazole or tinidazole therapy. Clinical isolates resistant to metronidazole can be resistant to tinidazole but usually with significantly lower minimal lethal concentrations to tinidazole than metronidazole [54, 55].

Conclusion

Molecular cell investigations, greatly facilitated by the availability of the genome sequence, have brought important new insights into TV pathobiology.

These developments combined with investigations on the microbiota and immunology of the urogenital tract have started to draw a more precise picture of the complex interplay between pathogens, the microbiota and the immune system. It is imperative to integrate these different data to improve our knowledge on human-microbe interactions to eventually improve our capacity to avoid and treat STIs that affect hundreds of millions of people of all ages, in particular those living in resource limited conditions.

Key points:

- Genomics, transcriptomics and proteomics datasets have started to boost research efforts characterising the molecular and cellular basis of TV pathobiology
- Complex interplay between TV, bacteria and viruses are being uncovered
- Future research efforts in the laboratory and the clinic will need to consider the complex interplay between TV, bacteria and viruses
- Diagnostic and clinical management of TV will benefit from basic research
- Improved diagnostic tests will enhance our understanding of the disease spectrum caused by TV

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Conflict of interest

There are no conflicts of interest.

Figure legends

Figure 1. Molecular phylogeny identify bird parasites as sister taxa to TV.

Molecular phylogeny based on an Rpb1 (the largest subunit of the RNA-polymerase II) protein alignment focusing on Trichomonadea are consistent with phylogenies derived from other genes - see Maritz et al. [5] for details including accession number of sequences. Species isolated from humans are highlighted with arrows; all other species were isolated from birds (bird species and country are indicated). Note one sequence derived from a bird isolate that is highly similar to TV sequences, which could represent a transfer from human to bird.

Figure 2. Host-TV interactions and its interplay with other microbes
A. Upon contact with human tissues (epithelial cells, extracellular matrix proteins [ECM]) TV trophozoites (pear-shaped cells) rapidly differentiate into amoeboid forms (pancake-like cells), dramatically increasing surface contact. This is one of the cellular processes thought to be essential for long-term infections [13, 20]. A combination of TV lipophosophglycans (LPG) and surface proteins mediate the binding to host tissues. Some TV proteins are delivered to the host cell surface via TV exosomes and thought to influence TV binding (see main text for these different points). B. Complex interplay between Bacteria, Viruses and Eukaryote in human-microbes interactions. The diagram illustrates a TV-infected pregnant woman and her partner in the global context of human-microbes interactions in their respective urogenital tracts. TV infections can affect the health status of both adults and the embryo (eg preterm birth and HIV transmission in utero). Other common eukaryotes encountered in the urogenital tract are Fungi of the genus *Candida*. TV, TVV, *Mycoplasma*, and other microbes can modulate the inflammatory status of the urogenital tracts and by doing can alter the susceptibility of a given individual to additional infectious agents such as HIV – see main text. Modified figure derived from [20].

Table 1. Some key features of the TV genome

Supplementary Digital content 1 (SDC1). Comparison of transcript abundance between two different TV strains for selected genes.

References and recommended reading

[1] WHO. Global incidence and prevalence of selected sexually transmitted infections - 2008. In: Geneva, Switzerland: World Health Organization; 2012. pp. 1-20.
[2] Carlton JM, Hirt RP, Silva JC *et al.* Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*. Science 2007; 315:207-212.
**[3] Conrad MD, Bradic M, Warring SD *et al.* Getting trichy: tools and approaches to interrogating *Trichomonas vaginalis* in a post-genome world. Trends Parasitol 2013; 29:17-25.
The latest review on the genome data and their exploitation through transcriptomics and proteomics to investigate the molecular and cellular basis of TV pathobiology. Include a discussion on the population genetics of TV and argue for the study of the parasite drug resistance using omics approaches.

[4] Carlton JM, Malik SB, Sullivan SA *et al.* The genome of *Trichomonas vaginalis*. In: Anaerobic Parasitic Protozoa: Genomics and Molecular Biology. Edited by: Clark CG, Johnson PJ, Adam RD. Caister Academic Press; 2010. pp. 45-77.
[5] Maritz JM, Land KM, Carlton JM, Hirt RP. What is the importance of zoonotic trichomonads for human health? Trends Parasitol 2014; 30:333-341.
[6] Palmer SR, Soulsby L, Torgerson P, Brown DWG. Oxford Textbook of Zoonoses, Biology, Clinical Practice, and Public Health Control. In: Second edition. Oxford University Press; 2011.
[7] Hirt RP, de Miguel N, Nakjang S *et al. Trichomonas vaginalis* pathobiology new insights from the genome sequence. Adv Parasitol 2011; 77:87-140.
[8] Ryan CM, de Miguel N, Johnson PJ. *Trichomonas vaginalis*: current understanding of host-parasite interactions. Essays Biochem 2011; 51:161-175.
[9] Figueroa-Angulo EE, Rendon-Gandarilla FJ, Puente-Rivera J *et al.* The effects of environmental factors on the virulence of *Trichomonas vaginalis*. Microbes Infect 2012; 14:1411-1427.
**[10] Gould SB, Woehle C, Kusdian G *et al.* Deep sequencing of *Trichomonas vaginalis* during the early infection of vaginal epithelial cells and amoeboid transition. Int J Parasitol 2013; 43:707-719.
**[11] Huang KY, Chen YY, Fang YK *et al.* Adaptive responses to glucose restriction enhance cell survival, antioxidant capability, and autophagy of the protozoan parasite Trichomonas vaginalis. Biochim Biophys Acta 2014; 1840:53-64.
The first two papers exploiting RNA-seq technologies to profile globally the mRNA concentration of all annotated genes. These papers pave the way to the future use of this approach to further rationalise the rather complex annotated proteome of the parasite.

[12] Noel CJ, Diaz N, Sicheritz-Ponten T *et al. Trichomonas vaginalis* vast BspA-like gene family: evidence for functional diversity from structural organisation and transcriptomics. BMC Genomics 2010; 11:99.
*[13] Kusdian G, Woehle C, Martin WF, Gould SB. The actin-based machinery of *Trichomonas vaginalis* mediates flagellate-amoeboid transition and migration across host tissue. Cellular microbiology 2013; 15:1707-1721.
New insights into the role of the actin cytoskeleton in regulating the amoeboid transformation of TV. The paper characterises the role of fimbrin in regulating the actin cytoskeleton and demonstrates a dramatic cellular redistribution of the protein

upon TV binding to VEC in vitro. A role of fimbrin in phagocytosis and cell migration is also presented.

*[14] Bradic M, Warring SD, Low V, Carlton JM. The Tc1/mariner transposable element family shapes genetic variation and gene expression in the protist *Trichomonas vaginalis*. Mob DNA 2014; 5:12.
An initial investigation of the potential role of TE in generating genetic and phenotypic diversity in TV. Insertions of one family of TE were shown to modulate the transcription of neighbouring genes. Based on these data the authors speculate that TE insertions could mediate adaptive evolution.

[15] de Miguel N, Lustig G, Twu O *et al.* Proteome analysis of the surface of *Trichomonas vaginalis* reveals novel proteins and strain-dependent differential expression. Mol Cell Proteomics 2010; 9:1554-1566.
[16] de Miguel N, Riestra A, Johnson PJ. Reversible association of tetraspanin with *Trichomonas vaginalis* flagella upon adherence to host cells. Cell Microbiol 2012; 14:1797-1807.
**[17] Twu O, de Miguel N, Lustig G *et al. Trichomonas vaginalis* exosomes deliver cargo to host cells and mediate host:parasite interactions. PLoS Pathog 2013; 9:e1003482.
An important new insight into the molecular and cellular basis of TV pathobiology. The paper demonstrates that TV secretes exosomes and characterise by proteomics their cargo proteins. By using in vitro experiments TV exosomes were shown to fuse to host cells and to modulate host-TV binding and inflammatory responses. The use of TV exosomes for diagnostic and as targets for local drug treatments is also suggested.

[18] Smith J, Garber GE. Current status and prospects for development of a vaccine against *Trichomonas vaginalis* infections. Vaccine 2014; 32:1588-1594.
**[19] Twu O, Dessi D, Vu A *et al. Trichomonas vaginalis* homolog of macrophage migration inhibitory factor induces prostate cell growth, invasiveness, and inflammatory responses. Proc Natl Acad Sci U S A 2014; 111:8179-8184.
Another important new insight in the molecular basis and cellular basis of TV pathobiology. The paper demonstrates that TV secretes a MIF homologue that modulates prostate cell growth and migration, suggesting that chronic TV infections could promote prostate cancers.

[20] Hirt RP. *Trichomonas vaginalis* virulence factors: an integrative overview. Sex Transm Infect 2013; 89:439-443.
**[21] Fiori PL, Diaz N, Cocco AR *et al.* Association of *Trichomonas vaginalis* with its symbiont *Mycoplasma hominis* synergistically upregulates the in vitro proinflammatory response of human monocytes. Sex Transm Infect 2013; 89:449-454.
One of several important publications that have initiated the characterisation of the complex interplay between TV, bacteria (Mycoplasma [21**, 24*], Lactobacilli [24**]) and TVV [23] in influencing host-parasite interaction by modulating the host inflammatory responses of VEC [23] or macrophages [21**] or inhibiting TV-VEC interactions [24**].

*[22] Fettweis JM, Serrano MG, Huang B *et al.* An emerging *Mycoplasma* associated with trichomoniasis, vaginal infection and disease. PLoS One 2014; 9:e110943.
A paper illustrating the value of metagenomic approaches to characterise the microbiota and pathogens of the urogenital tract. The data present the genome sequence of a novel *Mycoplasma* species that is strongly associated with TV infections.

[23] Fichorova RN, Lee Y, Yamamoto HS *et al.* Endobiont viruses sensed by the human host - beyond conventional antiparasitic therapy. PLoS One 2012; 7:e48418.
[24] Phukan N, Parsamand T, Brooks AE *et al.* The adherence of *Trichomonas vaginalis* to host ectocervical cells is influenced by lactobacilli. Sex Transm Infect 2013; 89:455-459.
*[25] Balkus JE, Richardson BA, Rabe LK *et al.* Bacterial vaginosis and the risk of *Trichomonas vaginalis* acquisition among HIV-1-negative women. Sex Transm Dis 2014; 41:123-128.
*[26] Brotman RM, Bradford LL, Conrad M *et al.* Association between *Trichomonas vaginalis* and vaginal bacterial community composition among reproductive-age women. Sex Transm Dis 2012; 39:807-812.
A selection of two, among several, papers investigating the association between TV infections and BV and how these might influence each other and impact negatively woman health.

[27] Swygard H, Sena AC, Hobbs MM, Cohen MS. Trichomoniasis: clinical manifestations, diagnosis and management. Sex Transm Infect 2004; 80:91-95.
[28] Cherpes TL, Wiesenfeld HC, Melan MA *et al.* The associations between pelvic inflammatory disease, *Trichomonas vaginalis* infection, and positive herpes simplex virus type 2 serology. Sex Transm Dis 2006; 33:747-752.
[29] Krieger JN, Jenny C, Verdon M *et al.* Clinical manifestations of trichomoniasis in men. Ann Intern Med 1993; 118:844-849.
[30] Seo MY, Im SJ, Gu NY *et al.* Inflammatory response of prostate epithelial cells to stimulation by *Trichomonas vaginalis*. Prostate 2014; 74:441-449.
[31] Mitchell L, Hussey J. *Trichomonas vaginalis*: an unusual presentation. Int J STD AIDS 2010; 21:664-665.
[32] Abdolrasouli A, Croucher A, Roushan A, Gaydos CA. Bilateral conjunctivitis due to *Trichomonas vaginalis* without genital infection: an unusual presentation in an adult man. J Clin Microbiol 2013; 51:3157-3159.
[33] Duboucher C, Noel C, Durand-Joly I *et al.* Pulmonary coinfection by *Trichomonas vaginalis* and Pneumocystis sp. as a novel manifestation of AIDS. Hum Pathol 2003; 34:508-511.
[34] Sutcliffe S. Sexually transmitted infections and risk of prostate cancer: review of historical and emerging hypotheses. Future Oncol 2010; 6:1289-1311.
[35] Cotch MF, Pastorek JG, 2nd, Nugent RP *et al. Trichomonas vaginalis* associated with low birth weight and preterm delivery. The Vaginal Infections and Prematurity Study Group. Sex Transm Dis 1997; 24:353-360.
[36] Cunnington M, Kortsalioudaki C, Heath P. Genitourinary pathogens and preterm birth. Current opinion in infectious diseases 2013; 26:219-230.
[37] Klebanoff MA, Carey JC, Hauth JC *et al.* Failure of metronidazole to prevent preterm delivery among pregnant women with asymptomatic *Trichomonas vaginalis* infection. N Engl J Med 2001; 345:487-493.

[38] Kigozi GG, Brahmbhatt H, Wabwire-Mangen F *et al.* Treatment of *Trichomonas* in pregnancy and adverse outcomes of pregnancy: a subanalysis of a randomized trial in Rakai, Uganda. Am J Obstet Gynecol 2003; 189:1398-1400.
[39] Bruins MJ, van Straaten IL, Ruijs GJ. Respiratory disease and *Trichomonas vaginalis* in premature newborn twins. Pediatr Infect Dis J 2013; 32:1029-1030.
[40] McClelland RS, Sangare L, Hassan WM *et al.* Infection with *Trichomonas vaginalis* increases the risk of HIV-1 acquisition. J Infect Dis 2007; 195:698-702.
[41] Mavedzenge SN, Pol BV, Cheng H *et al.* Epidemiological synergy of *Trichomonas vaginalis* and HIV in Zimbabwean and South African women. Sex Transm Dis 2010; 37:460-466.
[42] Van Der Pol B, Kwok C, Pierre-Louis B *et al. Trichomonas vaginalis* infection and human immunodeficiency virus acquisition in African women. J Infect Dis 2008; 197:548-554.
[43] Kissinger P, Mena L, Levison J *et al.* A randomized treatment trial: single versus 7-day dose of metronidazole for the treatment of *Trichomonas vaginalis* among HIV-infected women. J Acquir Immune Defic Syndr 2010; 55:565-571.
[44] Schwebke JR, Hobbs MM, Taylor SN *et al.* Molecular testing for *Trichomonas vaginalis* in women: results from a prospective U.S. clinical trial. J Clin Microbiol 2011; 49:4106-4111.
**[45] Munson E, Napierala M, Schell RF. Insights into trichomoniasis as a result of highly sensitive molecular diagnostics screening in a high-prevalence sexually transmitted infection community. Expert Rev Anti Infect Ther 2013; 11:845-863.
**[46] Hobbs MM, Sena AC. Modern diagnosis of *Trichomonas vaginalis* infection. Sex Transm Infect 2013; 89:434-438.
Two recent and detailed reviews on TV diagnostics developments and their impact on improving future control program and recognition of the importance of the parasite.

[47] Association of Public Health Laboratories. Advances in Laboratory Detection of *Trichomonas Vaginalis*. 2013:1-6. Available at: http://www.aphl.org/AboutAPHL/publications/Documents/ID_2013August_Advances-in-Laboratory-Detection-of-Trichomonas-vaginalis.pdf.
[48] Forna F, Gulmezoglu AM. Interventions for treating trichomoniasis in women. Cochrane Database Syst Rev 2003:CD000218.
[49] Hager WD, Brown ST, Kraus SJ *et al.* Metronidazole for vaginal trichomoniasis. Seven-day vs single-dose regimens. Jama 1980; 244:1219-1220.
[50] Thin RN, Symonds MA, Booker R *et al.* Double-blind comparison of a single dose and a five-day course of metronidazole in the treatment of trichomoniasis. Br J Vener Dis 1979; 55:354-356.
[51] Gulmezoglu AM, Azhar M. Interventions for trichomoniasis in pregnancy. Cochrane Database Syst Rev 2011:CD000220.
[52] Muzny CA, Schwebke JR. The clinical spectrum of *Trichomonas vaginalis* infection and challenges to management. Sex Transm Infect 2013; 89:423-425.
[53] Kirkcaldy RD, Augostini P, Asbel LE *et al. Trichomonas vaginalis* antimicrobial drug resistance in 6 US cities, STD Surveillance Network, 2009-2010. Emerg Infect Dis 2012; 18:939-943.
[54] Schwebke JR, Barrientes FJ. Prevalence of *Trichomonas vaginalis* isolates with resistance to metronidazole and tinidazole. Antimicrob Agents Chemother 2006; 50:4209-4210.

[55] Crowell AL, Sanders-Lewis KA, Secor WE. In vitro metronidazole and tinidazole activities against metronidazole-resistant strains of *Trichomonas vaginalis*. Antimicrob Agents Chemother 2003; 47:1407-1409.

Figure 1

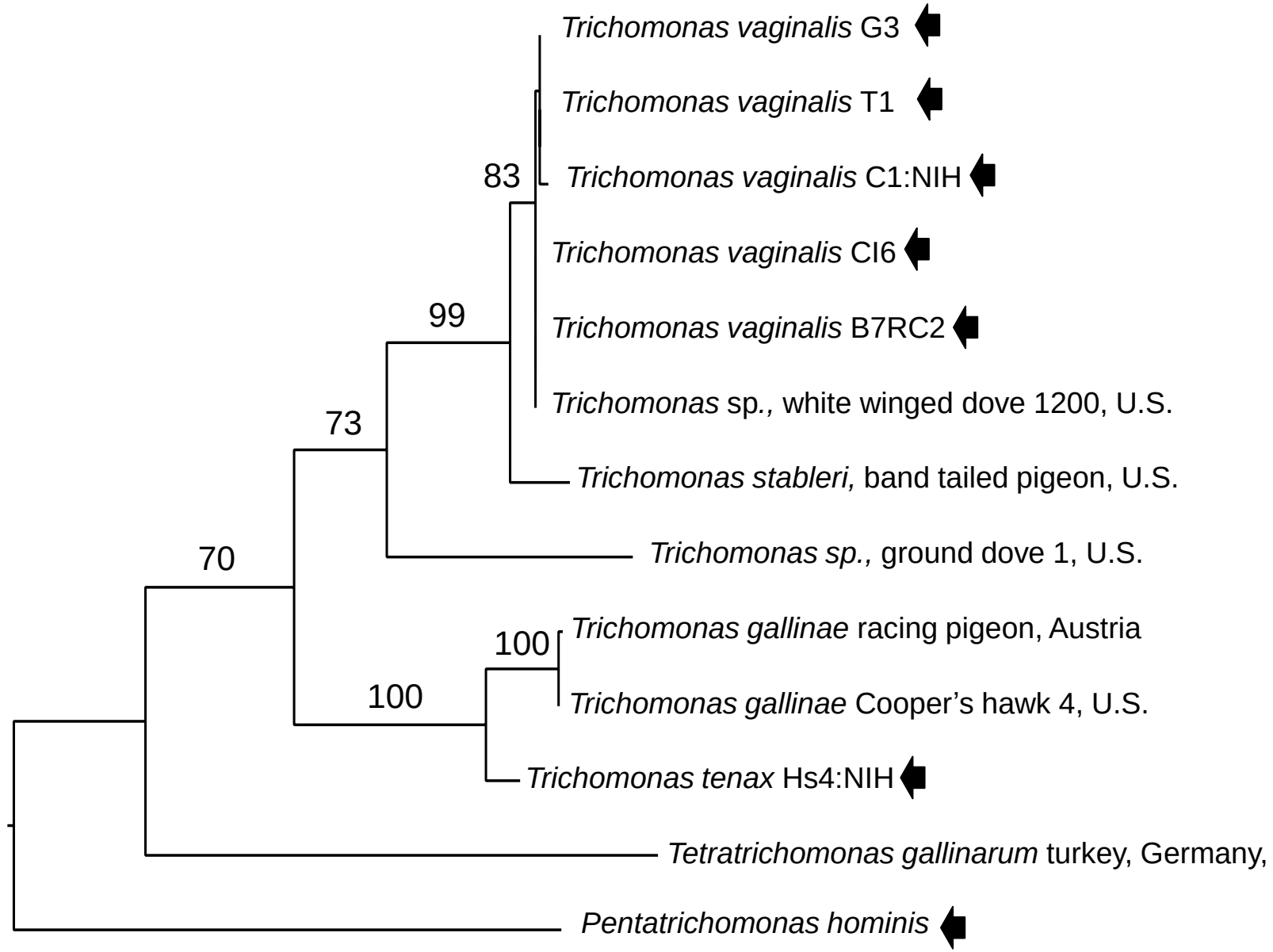
Trichomonas vaginalis G3
Trichomonas vaginalis T1
83
Trichomonas vaginalis C1:NIH
Trichomonas vaginalis CI6
99
Trichomonas vaginalis B7RC2
Trichomonas sp., white winged dove 1200, U.S.
73
Trichomonas stableri, band tailed pigeon, U.S.
70
Trichomonas sp., ground dove 1, U.S.
Trichomonas gallinae racing pigeon, Austria
100
100
Trichomonas gallinae Cooper's hawk 4, U.S.
Trichomonas tenax Hs4:NIH
Tetratrichomonas gallinarum turkey, Germany,
Pentatrichomonas hominis
0.08 Amino acids substitution/site

Figure 2

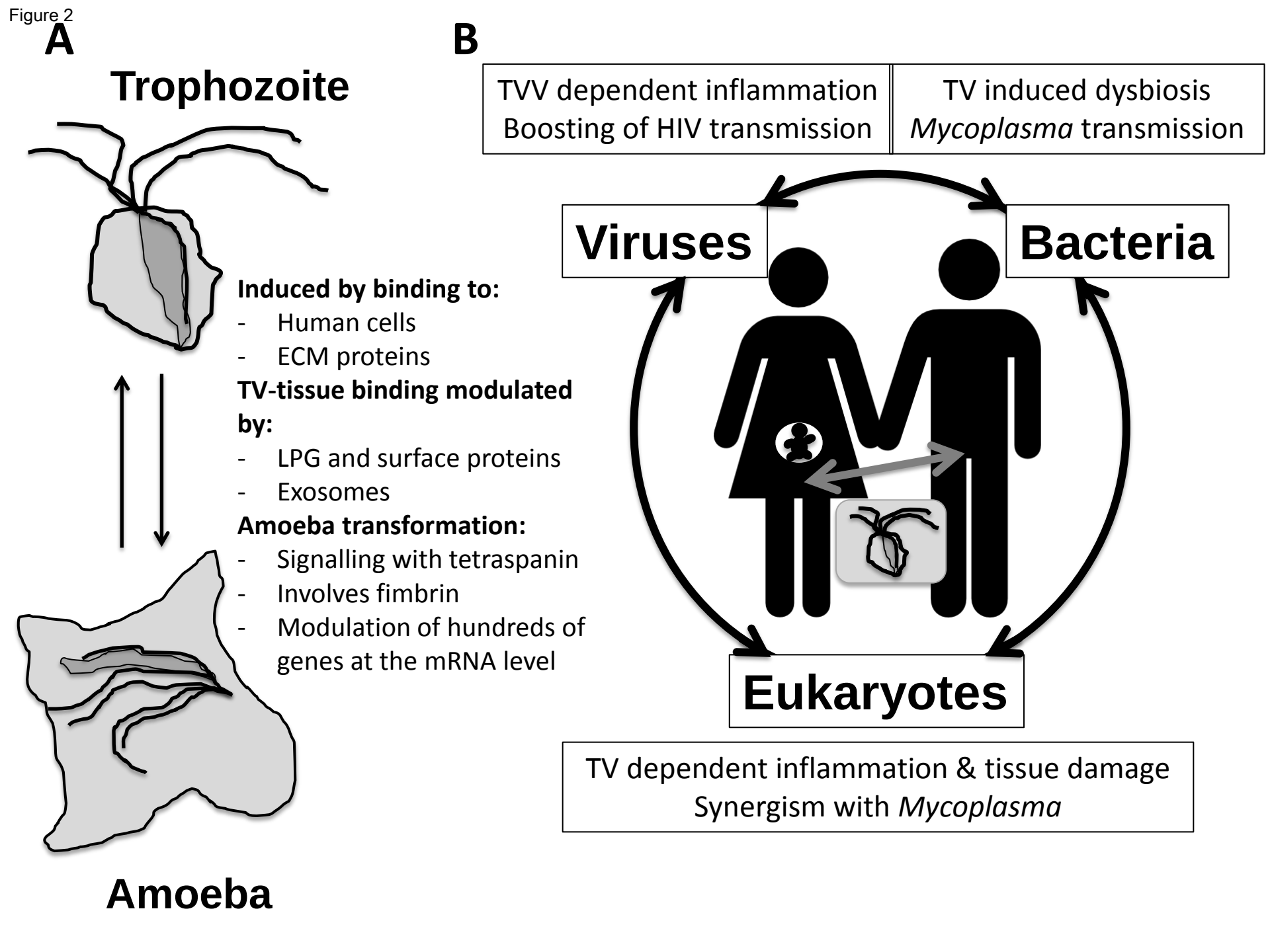
A
Trophozoite
Induced by binding to:
- Human cells
- ECM proteins
TV-tissue binding modulated by:
- LPG and surface proteins
- Exosomes
Amoeba transformation:
- Signalling with tetraspanin
- Involves fimbrin
- Modulation of hundreds of genes at the mRNA level
Amoeba
B
TVV dependent inflammation
Boosting of HIV transmission
TV induced dysbiosis
Mycoplasma transmission
Viruses
Bacteria
Eukaryotes
TV dependent inflammation & tissue damage
Synergism with Mycoplasma

Table 1. *Trichomonas vaginalis* genome features compared with selected species

Species	Predicted gene number (ORF)	Genome size (Mbp)	% of genome that is repetitive
*Trichomonas vaginalis**	59681	160	65
Homo sapiens	35845	2900	46
Drosophila melanogaster (fruit flie)	13679	180	2
*Entamoeba histolytica**	9938	25	6
*Giardia lamblia**	9649	12	?

Subset of a table from reference [2]
*Microbial eukaryotes infecting mucosal surfaces